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TI Steroid hormone receptor status defines the ***MMTV*** ***promoter*** chromatin structure in vivo.
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AU Xu A; Kudo S; Fukuda M
TI A novel expression vector composed of a regulatory element of the human leukosialin-encoding gene in different types of mammalian cells.
SO GENE, (1995 Jul 28) 160 (2) 283-6.
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TI The effect of various introns and transcription terminators on the efficiency of expression vectors in various cultured cell lines and in the mammary gland of transgenic mice.
SO JOURNAL OF BIOTECHNOLOGY, (1995 Jun 21) 40 (3) 169-78.
Journal code: AL6. ISSN: 0168-1656.

L5 ANSWER 15 OF 47 MEDLINE DUPLICATE 10
AU Wilson S E; Weng J; Blair S; He Y G; Lloyd S
TI Expression of E6/E7 or SV40 large T antigen-coding oncogenes in human corneal endothelial cells indicates regulated high-proliferative capacity.
SO INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, (1995 Jan) 36 (1) 32-40.
Journal code: GWI. ISSN: 0146-0404.

L5 ANSWER 17 OF 47 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 12
AU Pendse, Girish J.; Bailey, James E.
TI Effect of Vitreoscilla hemoglobin expression on growth and specific tissue plasminogen activator productivity in recombinant Chinese hamster ovary cells
SO Biotechnol. Bioeng. (1994), 44(11), 1367-70
CODEN: BIBIAU; ISSN: 0006-3592

L5 ANSWER 18 OF 47 MEDLINE DUPLICATE 13
AU Archer T K; Zaniewski E; Moyer M L; Nordeen S K
TI The differential capacity of glucocorticoids and progestins to alter chromatin structure and induce gene ***expression*** in ***human*** breast cancer ***cells*** .
SO MOLECULAR ENDOCRINOLOGY, (1994 Sep) 8 (9) 1154-62.
Journal code: NGZ. ISSN: 0888-8809.

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# Steroid Hormone Receptor Status Defines the MMTV Promoter Chromatin Structure *In Vivo*

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**The ability to respond to small signalling molecules such as steroid hormones is important for many physiological processes. Steroid hormones act through a group of high affinity receptors that regulate transcription by binding to hormone response elements (HREs) located within the promoters of target genes, which themselves are organized with nuclear proteins to form chromatin. To dissect the mechanisms(s) of steroid hormone action we have used the steroid inducible mouse mammary tumor virus (MMTV) promoter as a model system. The MMTV promoter is assembled into a phased array of nucleosomes that are specifically positioned in rodent cells. Induction of transcription by glucocorticoids is accompanied by the appearance of a hypersensitive region in the proximal promoter which allows the hormone dependent assembly of a preinitiation complex including transcription factors such as nuclear factor 1 (NF1) and the octamer transcription factor (OTF). Surprisingly, when introduced by transient transfection, the progesterone receptor (PR) is unable to activate this promoter *in vivo*, a finding that may result from its inability to alter MMTV promoter chromatin. In an attempt to investigate the failure of the PR to activate the promoter, we have stably introduced the MMTV promoter into human T47D breast cancer cells that express high levels of the PR. In contrast to what has been observed previously in rodent cells, the MMTV templates resident in human breast cancer cells adopt a novel and constitutively open chromatin structure. The constitutively open chromatin structure is accompanied by the hormone independent loading of transcription factors including the PR and NF1. In T47D cells that stably express the glucocorticoid receptor, the MMTV promoter responds to glucocorticoids, but not progestins, and displays glucocorticoid induced restriction enzyme hypersensitivity and transcription factor loading. These findings suggest that the organization of the MMTV chromatin structure is dependent upon the cell type and receptor status of the recipient cell into which the MMTV promoter is stably introduced.**

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## INTRODUCTION

During the last several years a significant amount of new information has demonstrated that the underlying architecture of DNA as chromatin may have a significant impact on the regulation of transcription and contribute to disease processes [1–3]. The role of chromatin and nuclear proteins in packaging of DNA to allow compaction within the eukaryotic nucleus is well established [4, 5]. Approximately 146 bp of DNA is intimately associated with 8 histone core proteins to form a mononucleosome [6–8]. This repeating structure forms the basis of all the higher chromatin structures and also allows the association of linking proteins, such as histone H1, and non-histone chromatin proteins such as the HMG proteins [4, 9]. Genetic analysis with simple organisms such as *Saccharomyces cerevisiae*, combined with powerful biochemical approaches analyzing mammalian and avian histone proteins, have provided *in vivo* and *in vitro* experimental avenues to characterize this process in detail [1, 2, 10–12].

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Characterization of chromatin structure in cell lines derived from tissues of humans and other mammals has also significantly advanced our understanding of how chromatin can regulate the access of transcription factors to their cognate binding sites and thereby influence both basal and activated transcription [13–15]. Studies utilizing the mouse mammary tumour virus long terminal repeat (MMTV LTR) have been invaluable for studying the mechanisms by which the chromatin environment influences transcription factor access in general and specifically the regulation of transcription by steroid hormones [16–22].

Beginning with a series of studies in which the MMTV LTR was stably introduced into rodent cells, it was demonstrated, by a variety of techniques, that the LTR acquired a phased array of 6 nucleosomes (Fig. 1) [23]. This was the case whether the LTR was introduced as a viral integrant, in a multicopy bovine papilloma virus (BPV) episomal vector or stably integrated into the chromosome via transfection. The proximal portion of the LTR corresponding to the second or "B" nucleosome of the phased array exhibits significant hypersensitivity to restriction enzymes and agents such as DNase 1 when cells are treated with glucocorticoids (Fig. 1) [23, 24]. This modification or displacement of nucleosome B (Nuc-B), which encompasses the hormonal response elements (HREs; Fig. 1), is a signature of the hormone response from this promoter (Fig. 2) [25]. This hypersensitivity is a prelude to the formation of an active, pre-initiation complex that includes transcription factors nuclear factor-1 (NF1), octamer transcription factor (OTF), the TATA binding protein (TBP) and a recently described factor $F_{DT}$ (factor downstream of TBP, see below) [16, 18, 19] (Fig. 2). These experiments led to the proposal that the organization into chromatin contributes mechanistically to glucocorticoid regulation of transcription from the MMTV LTR [18].

The impact of chromatin structure on transcriptional regulation by steroid hormones *in vivo* was examined using transiently transfected MMTV templates in cell lines that stably maintain copies of the MMTV promoter [26–28]. Analysis of transiently introduced copies of the MMTV promoter did not detect an ordered array of nucleosomes [18, 26]. Indeed, the transiently introduced template was constitutively hypersensitive and NF1 was bound in the presence or absence of hormone [18]. The mechanism by which the NF1 bound promoter remained transcriptionally inactive was resolved by additional experiments showing that TBP was only detected in the presence of hormone [18, 26].

Previous *in vitro* experiments [17] have allowed us to sub-divide transacting factors into those to which the DNA is "transparent" when in a nucleosome [29], i.e. the glucocorticoid receptor (GR), and those where the sites are "opaque" and thereby prevented from binding (i.e. NF1, OTF, TBP and $F_{DT}$). Our *in vivo* footprinting experiments, comparing transiently transfected and stable chromatin templates [18, 19, 30], allow us to further sub-divide the second category into two groups. The first group of proteins (i.e. NF1 and OTF) are able to interact with their sites provided they are not assembled as chromatin *in vivo* [27, 30]. The second group of factors (i.e. TBP and $F_{DT}$) require the action of ancillary factors and/or the GR to occupy their sites on both chromatin and non-chromatin templates [19].

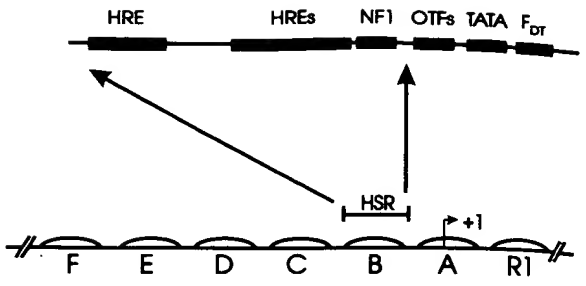


**Fig. 1. Schematic representation of the nucleosomal organization of the MMTV LTR. The MMTV LTR is reproducibly assembled into a phased array of six nucleosomes (A–F) when stably introduced into cells [23]. The region occupied by nucleosome B (−221 to −75) coincides with the hormone inducible hypersensitive region (HSR) [23, 24]. The proximal promoter region contains binding sites for the steroid receptors (HRE), nuclear factor 1 (NF1), the octamer transcription factors (OTFs), the TATA binding protein (TBP) and a factor binding downstream of the TBP ($F_{DT}$).**

These experiments also provided the first evidence for hormone independent binding of the OTFs (both distal and proximal) and hormone dependent binding of $F_{DT}$ to the MMTV LTR *in vivo*. This latter factor, which binds between the TATA box and the transcrip-

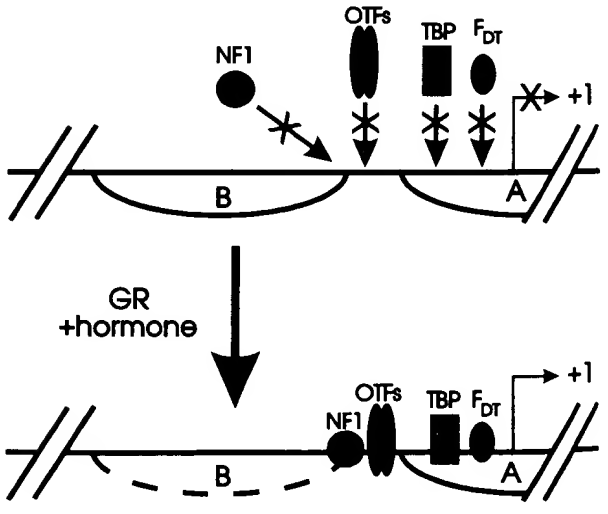


**Fig. 2. Model of hormone induced transcription from the MMTV LTR. The nucleosomal architecture of the MMTV LTR excludes binding of the transcription factors NF1, OTFs, TBP, and $F_{DT}$ prior to glucocorticoid treatment. Hormone activation of the GR results in an altered organization of nucleosome B (indicated by the dotted line) that permits loading of NF1 and the assembly of the transcription initiation complex [16, 18].**

tion initiation start site ( − 10 to − 24), is also found in a variety of cell types in addition to the mouse mammary cells where it was first identified (T. Liang and T. K. Archer, unpublished data). Physical characterization of the $F_{DT}$ protein, by Southwestern and UV cross-linking assays with the $F_{DT}$ binding site, are currently underway.

## CHROMATIN STRUCTURE INFLUENCES TRANSCRIPTION PREINITIATION COMPLEX DISASSEMBLY AS WELL AS ASSEMBLY

The induction of transcription by glucocorticoids is rapid, but transient, such that mRNA initiation peaks by 1 h and declines to basal levels by 24 h [31]. *In vivo* restriction enzyme hypersensitivity assays demonstrated that similar kinetics were evident for the hormone induced changes in chromatin structure. Restriction enzymes exhibited enhanced cleavage, of sites within the Nuc-B region of the MMTV LTR, at 1 h, but not after 24 h of glucocorticoid treatment. An examination of the kinetics of transcription factor loading onto the MMTV LTR revealed NF1, OTF and TBP binding after 1 h, but not 24 h of hormone treatment. Thus the significant reduction in transcription from the MMTV LTR observed after 24 h of hormone treatment correlates with a loss of chromatin hypersensitivity and the failure to detect the transcription initiation complex [19].

This rapid de-induction occurs in the presence of active hormone and suggests that some feature(s) of chromatin remodelling require(s) an activity or protein that becomes unavailable for the reactivation of this promoter [19]. Previous experiments have addressed the initial events of transcription, with emphasis on the hypersensitivity of the promoter to cleavage by restriction enzymes [17] and the formation of the preinitiation complex [16]. We have now conducted a series of experiments designed to determine the role of chromatin structure in the termination of transcription [19]. These experiments were designed to determine if this phenomena could be due either to a chromatin transition (template related), or involve receptor cycling or modification (receptor related) [27].

To examine a template related model we used a polymerase chain reaction coupled exonuclease III (PCR/Exo III) *in vivo* footprinting strategy that distinguishes transient from stable templates within the same cells [18, 26]. For chromatin templates, glucocorticoid stimulation lead to the assembly of the preinitiation complex containing NF1, OTF, TBP and $F_{DT}$ at 1 h, but not at 24 h [19]. The results with the transient template were dramatically different as NF1 and OTF were bound under all conditions. However, TBP and $F_{DT}$ were only observed in the presence of dexamethasone both at 1 and 24 h. The hormone dependent loading of TBP and $F_{DT}$ on the transient template, at times when the stable template is refractory, is consistent with the presence of active receptor in the nucleus. Furthermore, when reporter plasmids are transiently introduced into cells previously treated with dexamethasone for 24 h and exhibiting no transcription from the endogenous template, they are active [19]. These experiments demonstrate that the activities of NF1, OTF, TBP, GR and $F_{DT}$ were neither down-regulated nor diminished by prolonged exposure to hormone, thus supporting a template model for the observed MMTV kinetics. They also link changes in chromatin organization of the promoter to the cessation of transcription in a manner similar to that shown previously for the initiation of transcription.

## PROGESTERONE REGULATION OF MMTV TRANSCRIPTION IN HUMAN BREAST CANCER CELLS

While the above studies have focused on glucocorticoid induction of transcription, the MMTV LTR also responds to progestins, androgens and mineralocorticoids [20, 32–34]. As the receptors for these hormones recognize identical HREs *in vitro*, it is not immediately obvious how a selective hormone response is achieved *in vivo* [35]. Evidence for a diverse group of mechanisms have been put forward recently. Selectivity can be achieved enzymatically, as exemplified by the tissue-specific expression of 11$\beta$-dehydrogenase, which permits selective response to mineralocorticoids in an environment where levels of glucocorticoids would saturate the mineralocorticoid receptor [36]. For the proliferin promoter, which contains a composite regulatory element, the differential interaction of non-receptor factors with specific hormone receptor domains determines the specificity of response between the highly related GR and mineralocorticoid receptors [37, 38]. Similarly, androgen specific responses may be mediated by cooperative interactions between factors binding to auxiliary DNA elements and the androgen receptor [39, 40]. Finally, the receptor complement itself clearly influences selectivity, as the introduction of the progesterone receptor (PR) into hepatoma cells confers progesterone responsiveness to the endogenous glucocorticoid-regulated tyrosine amino transferase (TAT) gene [15].

We have recently suggested that the organization of genes into specific chromatin structures may also modulate the activity of hormone receptors in specific cells and tissues. The experimental basis for this hypothesis was our observation that transient introduction of a PR expression vector into pr− cells is sufficient to activate a non-chromatin transiently introduced MMTV LTR, but not the endogenous MMTV template organized as chromatin [31, 41]. In contrast, glucocorticoids stimulate transcription from both transient and stable templates [31]. This result suggests that unlike the GR, the PR may not be able to induce the chromatin transition and loading of NF1 required

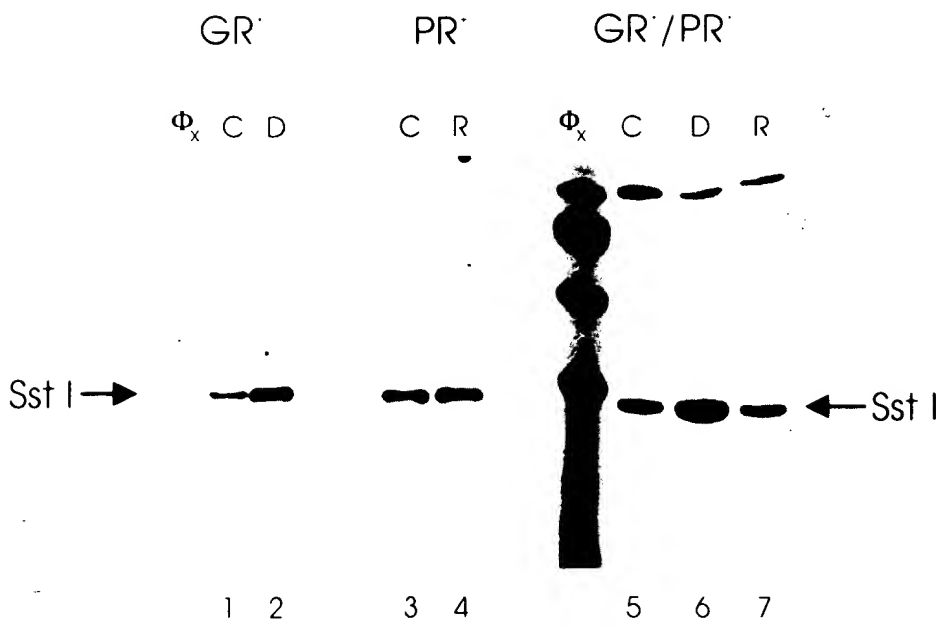


**Fig. 3. Comparison of restriction enzyme hypersensitivity of the MMTV LTR in cells with different receptor complement. GR+ mouse 1471.1 cells treated with dexamethasone show enhanced cleavage by Sst I over untreated control cells (cf. lanes 1 and 2). PR+ human T47D/2963.1 cells display constitutive hypersensitivity to cleavage by Sst I in the presence or absence of R5020 (cf. lanes 3 and 4). GR+/PR+ human T47D/A1–2 cells show enhanced cleavage by Sst I when treated with dexamethasone, but not when treated with R5020 (cf. lanes 6 and 7 with lane 5). Lanes 1–2, 1471.1 cells; lanes 3–4, T47D/2963.1 cells; lanes 5–7, T47D/A1-2 cells. C, control; D, dexamethasone; R, R5020. $\phi_X$, PhiX174 RF DNA digested with Hae III.**

for activation of transcription from a MMTV template incorporated into chromatin. PR activation of the transient template still occurs as these templates are not organized in a phased array of nucleosomes and have NF1 bound both in the presence and absence of hormone [18]. Thus, it appears that the specific organization of the template as chromatin in these cells is refractory to stimulation by newly introduced PR.

To facilitate a detailed analysis of the actions of endogenous PR on a stably maintained template, we stably introduced the MMTV LTR into T47D breast cancer cells, which naturally express a high level of both the $PR_A$ and $PR_B$ isoforms and a non-functional GR [34]. One of the clones selected for analysis, designated 2963.1, contains 10 copies of the MMTV LTR per cell [42]. Chloramphenicol acetyltransferase (CAT) assays and primer extension analysis showed that expression from the stably maintained chimeric MMTV/CAT gene in 2963.1 cells was induced 8–10-fold by the synthetic progestin R5020 and this induction was blocked by the progesterone antagonist RU486 [42]. As compared to previous analysis [43], the relatively low fold induction by R5020 in these cells likely results from a high basal level of expression and the novel chromatin organization of the MMTV LTR in these cells (see below).

In mouse cells, the MMTV LTR is reproducibly assembled into a precisely positioned phased array of nucleosomes *in vivo* (Fig. 1), whether stably integrated, maintained episomally, or when assembled *in vitro* [17, 25]. A comparison of the organization of the MMTV LTR organization in human T47D derived 2963.1 cells with that of the extensively characterized 1471.1 mouse cell line using a high resolution micrococcal nuclease digestion analysis revealed identical positioning of Nuc-B and Nuc-A in the two cell lines [42].

In GR+ mouse cells, the MMTV LTR nucleosomal array undergoes a structural transition upon glucocorticoid treatment, such that enhanced cleavage (hypersensitivity) is observed by restriction enzymes with cleavage sites within the region occupied by Nuc-B [17] (Fig. 3, lanes 1 and 2). Surprisingly, no such changes in chromatin structure were produced upon progestin treatment of human PR+ 2963.1 cells [42] (Fig. 3, lanes 3 and 4). This result was unrelated to the low copy number in 2963.1 cells, as restriction enzyme hypersensitivity was observed in the GR+ 2305 cell line, which contains a similarly low copy number (15 vs 10 copies) [43]. In the absence of hormone, the extent of cleavage by restriction enzymes recognizing sites within Nuc-B was considerably greater in 2963.1 cells than in mouse cells (Fig. 3, cf. lanes 1 and 3). This suggested that even in the absence of hormone, the MMTV LTR in the 2963.1 cells has already adopted an open conformation approximating that observed for transiently introduced non-chromatin templates [18, 19]. These results contrast with the micrococcal nuclease analysis, which showed that the organization of Nuc-B in 2963.1 cells was the same as for that in GR+ mouse cells. This may not be surprising, as analogous experiments in mouse cells fail to detect any

changes in micrococcal nuclease cleavage upon hormone stimulation (T. K. Archer, unpublished data and [44]).

The surprising observation that the region encompassed by Nuc-B was constitutively accessible to cleavage by restriction enzymes prompted us to examine the organization of other regions of the LTR. Restriction enzyme cleavage within the regions of the MMTV LTR incorporated into the adjacent Nuc-A and Nuc-R1 region was inefficient in 2963.1 cells. In contrast, cleavage at these sites was very efficient in a transiently introduced non-chromatin template [42]. Thus, while the organization of Nuc-B in the stable chromatin template in 2963.1 cells approximates that of a transiently introduced non-chromatin template, the rest of the promoter differs in that it appears to be organized into a "repressive" chromatin architecture similar to that observed in pr−/GR+ cells.

To evaluate the consequences of the constitutive hypersensitivity observed in the proximal promoter of the MMTV LTR in 2963.1 cells on access of transcription factors, we employed an *in vivo* PCR/Exo III footprinting assay. In sharp contrast to what we have observed previously in GR+ containing cells lines [16, 45], we detected significant binding of NF1 and OTF to the MMTV LTR in non-stimulated as well as hormone treated 2963.1 cells [30, 43] (Fig. 4). Interestingly, additional hormone independent exonuclease stops were observed in 2963.1 cells [42], which coincide with the 5′ boundary of proximal HRE [46] and the binding sites for factor(s) present in mouse cell extracts that were previously shown to bind the MMTV LTR *in vitro* [47] (Fig. 4).

Our ability to detect binding by the PR, but not the GR by *in vivo* footprinting may reflect differences in the modes of action of these two receptors [31, 41]. We have investigated a consequence of this difference by examining the kinetics of the hormone response in 2963.1 cells. As mentioned above, glucocorticoid induction of transcription from chromatin copies of the MMTV LTR is short lived, returning to basal levels within 24 h [19, 31]. This cessation of transcription has been correlated with a reformation of chromatin architecture into a "repressive" state and is not observed for non-chromatin MMTV templates, arguing that the chromatin structure of the LTR provides a mechanism for the disassembly of the transcription pre-initiation complex [19]. As the constitutively open chromatin organization we observe in 2963.1 cells approximates that observed for non-chromatin templates, we anticipated that the kinetics of hormone response in these cells would be similar to that observed for a non-chromatin template. Restriction hypersensitivity analysis and *in vivo* Exo III footprinting experiments demonstrate elevated cleavage and binding by NF1 and the PR even after 24 h of hormone treatment [42]. These results differ greatly from those performed on stable chromatin templates in GR+ cells, but are similar to those from transiently introduced templates [19]. Thus these data provide additional confirmation of the constitutively open chromatin organization of the MMTV LTR in 2963.1 cells and further support a role for a repressive chromatin structure in the cessation of transcription from the MMTV LTR.

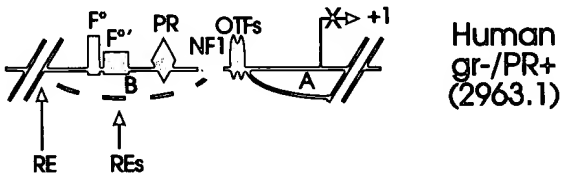


**Fig. 4. Transcription factor loading and restriction enzyme hypersensitivity of the MMTV LTR in PR+ human T47D cells. In human T47D/2963.1 cells, which contain the PR but not a functional GR, nucleosome B is organized into a constitutively open architecture (as indicated by the dotted line) [42]. Transcription factors including the PR, NF1 and OTFs bind to the LTR even in the absence of hormone treatment, and this region exhibits constitutively hypersensitive restriction enzyme cleavage [42].**

Our analysis of the 2963.1 cell line suggests that the novel chromatin organization we observe may reflect the occupancy of the MMTV LTR by transcription factors such as the PR and NF1. This occupancy could compete with chromatin assembly following DNA replication, resulting in the altered nucleosomal structure we observe. It has been suggested that nucleosome assembly and binding of at least some transcription factors may be mutually exclusive [28]. Such a competition has been observed for the 5S RNA gene of *Xenopus* [48], in which transcription factors required for constitutive transcription of the somatic 5S rRNA form stable complexes that prevent subsequent chromatin maturation. Furthermore, in a situation that is similar to that reported here, *in vivo* footprinting experiments with the serum albumin gene demonstrate that transcription factor binding leads to the assembly of a specific chromatin structure during liver specific transcription [49]. Our observation that Nuc-B of the MMTV LTR is constitutively organized into a more open conformation in these PR containing cells is significant, as it provides new insight into mechanisms by which the receptor and non-receptor complement of a given cell can act to determine the chromatin organization of target genes, and hence their ability to respond to a variety of signals.

In a related series of experiments we have analyzed the consequences of co-expression of the PR and GR on the regulation and chromatin organization of the MMTV LTR in human T47D cells. The A1–2 cell line was constructed to express comparable levels of GR and PR [34], and contains 10 stably integrated copies of a MMTV Luciferase chimera [50]. Analysis of hormone mediated induction of MMTV transcription in the A1–2 cell line revealed a marked difference between the abilities of glucocorticoids and progestins

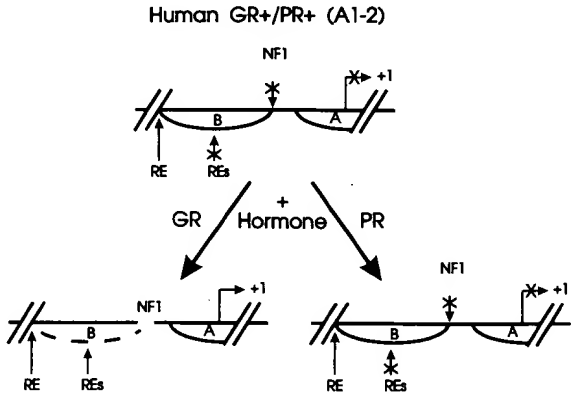


**Fig. 5. Transcription factor loading and restriction enzyme hypersensitivity of the MMTV LTR in PR+/GR+ human T47D cells. In human T47D/A1-2 cells, which contain the PR and GR, nucleosome B is organized into a closed architecture prior to hormone addition [50]. This organization excludes NF1 binding and limits the access of restriction enzymes. Treatment with glucocorticoids, but not progestins, results in a change in the organization of nucleosome B (indicated by the dotted line) that permits loading of NF1 and allows efficient access by restriction enzymes [50].**

to induce transcription from the endogenous MMTV reporter. Unlike the gr−/PR+ 2963.1 cells [42], induction of transcription from the stably maintained MMTV LTR in these cells by progesterone or R5020 was barely measurable. In contrast, induction by dexamethasone was 150-fold higher than that observed for progestins [50]. This disparity between glucocorticoid and progestin mediated induction is also observed for induction of expression from an otherwise identical transiently introduced non-chromatin template, although in this case the difference is only 20-fold [34].

*In vivo* restriction enzyme hypersensitivity analysis of the stably maintained MMTV LTR in A1–2 cells demonstrated that cleavage by restriction enzymes within the region encompassed by Nuc-B was inefficient in the absence of either dexamethasone or R5020 [50] (Fig, 3, lane 5). This immediately suggested that unlike the gr−/PR+ 2963.1 cells, the MMTV LTR in these cells was organized into the "closed" conformation observed previously in GR+ mouse cells. The addition of dexamethasone, but not R5020, enhanced cleavage by restriction enzymes with recognition sites within Nuc-B [50] (Fig. 3, lanes 6 and 7). Thus, the GR but not the PR is able to induce the transition from "closed" to "open" chromatin [50] (Fig. 5).

Using an *in vivo* PCR/Exo III footprinting assay, we demonstrated that dexamethasone induced hypersensitivity is accompanied by NF1 loading on the MMTV LTR, while progestin treated cells displayed only a low level of NF1 loading (Fig. 5) [50]. In subsequent experiments we studied the effects of simultaneously administering both dexamethasone and progestin to A1–2 cells. The coadministration of dexamethasone and progestin for 6 h resulted in a greater than 5-fold inhibition of the dexamethasone-induced response. Furthermore, when dexamethasone and R5020 are added concurrently, restriction enzyme hypersensitivity induced by dexamethasone is reduced [50]. The mechanism by which the activated PR interferes with GR action is currently under investigation.

These experiments on A1–2 cells strongly suggest that receptor complement plays a central role in establishing the chromatin organization of target genes. When both the GR and PR are present in T47D cells, only glucocorticoids can activate transcription from MMTV sequences assembled as chromatin [50]. This result was surprising, as PR mediated activation of transcription from a chromatin MMTV template clearly occurs in the absence of a functional GR in 2963.1 cells [42]. The explanation for this result appears to lie within the differences in chromatin organization of the MMTV LTR between these two T47D derived cell lines. In gr−/PR+ 2963.1 cells, the MMTV LTR is constitutively "open", while in GR+/PR+ A1–2 cells the MMTV LTR is "closed" prior to hormone stimulation. Interestingly, in reciprocal experiments where chicken $PR_A$ was stably expressed in a GR+ mouse cell line progestins activated transcription from the MMTV LTR [41]. This difference may result from species specific activities of chicken and human PR or functional differences between the $PR_A$ and $PR_B$ isoforms [51–53]. Nevertheless, the receptor complement of the cell plays an important role in regulating steroid hormone regulation of transcription.

## CONCLUSIONS

The data reviewed here clearly demonstrate the intimate relationship between transcription and the chromatin organization of the transcribed gene. Using the MMTV LTR as our model system, we have shown that the assembly of this promoter into a phased array of nucleosomes acts "statically" to repress basal transcription by restricting access of ubiquitously available transcription factors. The results from recent studies suggest that the MMTV LTR chromatin structure is dynamic and the precise arrangement of nuclear proteins is dependent upon the cellular environment, including the class of nuclear receptor expressed [27]. How then is transcription regulated at the MMTV promoter? Our results strongly suggest that the exclusion of NF1 and OTF from high affinity sites can be attributed directly to the organization of these sites as chromatin. The loading of TBP and $F_{DT}$ onto the promoter requires the GR to facilitate binding, but it is not known whether this recruitment of TBP and/or $F_{DT}$ is direct or indirect.

Despite the fact that the GR is absolutely required to initiate this cascade of events, footprinting experiments have not reproducibly detected the bound GR. We have proposed that this may be due to the GR:chromatin association being labile to Exo III or

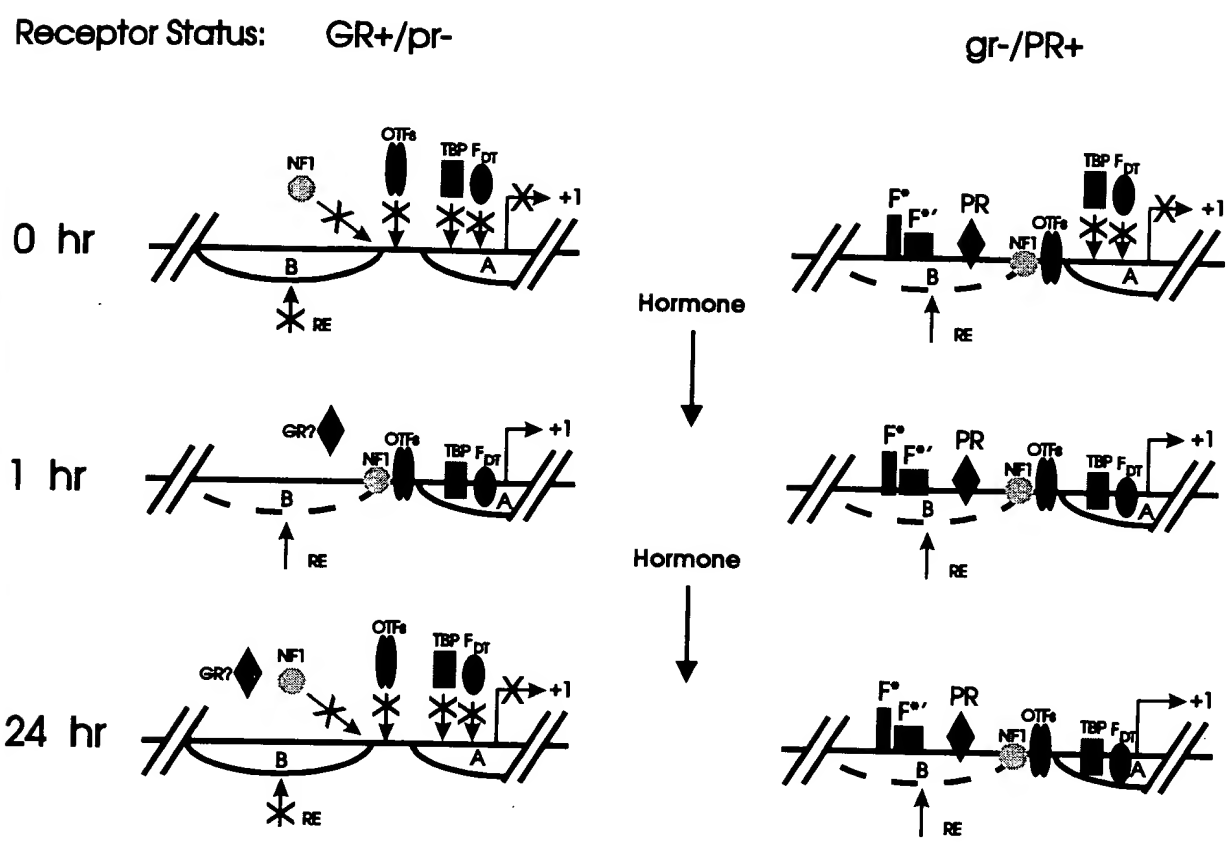


Fig. 6. Steroid receptor status and chromatin organization: mechanistic implications. A model is presented for the mechanisms by which the glucocorticoid and progesterone receptors act to regulate transcription from chromatin templates *in vivo*. The left panel depicts GR induction in cells that are GR+/pr−. The right panel depicts PR induction in cells that are gr−/PR+. See text for details.

that its mechanism of action involves a transient interaction with the template [25]. A similar proposal has been made for the TAT gene [54]. Support for the idea of a transient interaction comes from our experiments with human T47D/2963.1 cells [30, 42]. In this case the promoter is always hypersensitive and a group of transcription factors, including the PR, are always present on the template. It is reasonable to expect that the affinity of the GR and PR for DNA would be almost identical given the homology of their DNA binding domains [35]. The ability to detect the PR, but not the GR, suggests that the GR is not stably present on the template even in the presence of hormone [42]. If this were correct, it would argue that the kinetics of GR induced transcription and chromatin remodelling result from this transient GR:chromatin interaction.

Our experiments suggest a model for the mechanisms by which the glucocorticoid and progesterone receptors act to regulate transcription from chromatin templates *in vivo* (Fig. 6). In the absence of hormone GR+ and gr− cells display distinct chromatin organizations. For the GR+ cells transcription factors are excluded from the promoter, while in the PR+ cells the promoter is bound by a variety of proteins including NF1, OTF and the PR. During the initial phase of the induction by glucocorticoids, +hormone for 1 h, the MMTV promoter acquires an identical complement of transcription factors including TBP. However, at 24 h of hormone treatment there is a loss of the pre-initiation complex that is observed in GR+ mouse cells and the subsequent re-establishment of the repressive chromatin structure [19, 32]. This may result from the dissociation of the GR from the promoter leading to a "hit and run" model of GR activation. In contrast, the stable binding of the PR in PR+ cells would inhibit the "re-chromatinization" of the B nucleosome and NF1 would remain bound at 24 h [42]. While we know little of the mechanism by which the pre-initiation complex is lost, it clearly results from "re-chromatinization" rather than down regulation of transcription factors [19]. Thus the differences observed would be related to receptor content of the cell line examined and suggest that the steroid hormone receptor status defines the MMTV promoter chromatin structure *in vivo*.

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